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MEMORANDUM

SUBJECT: ***THIOPHANATE-METHYL*** - **REVISED** Report of the Hazard Identification Assessment Review Committee based on the September 26, 2000 meeting.

FROM: John Doherty
ReRegistration Branch III
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chair
and
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Steve Knizner, Senior Scientist
Re-Registration Branch 3
Health Effects Division (7509C)

PC Code: 102001

On **September 26, 2000,** the Hazard Identification Assessment Review Committee (HIARC) evaluated the results of a rabbit developmental toxicity study that was recently submitted to the Agency and its impact on FQPA assessment and the toxicity endpoint selection. This study conducted in 1997 was requested by the Agency to verify the equivocal findings seen in the 1986 rabbit developmental toxicity study.

In addition, the HIARC also reconsidered the acceptability status of the rat developmental dietary toxicity study based on the recommendation of HED's Toxicology Scientific Advisory Council (TOX SAC) for completeness of the developmental toxicity data base. **The impact of accepting this new study has resulted in revisions to the toxicity endpoint selected for dietary and non-dietary exposure scenarios as well as the FQPA assessment for increased susceptibility to infants and children.**

This report supercedes the HIARC Reports issued April 27, 1999 and December 16, 1999.

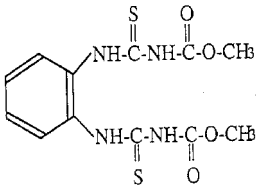
Committee Members in Attendance

Members present were: Ayaad Assad, William Burnam, Jonathan Chen, Elizabeth Doyle, Pamela Hurley, Tina Levine, David Nixon, Yung Yang, and Jess Rowland.

Committee members *in absentia* were: Elizabeth Mendez

Data Presentation: and Report Presentation	John Doherty Toxicologist

I. INTRODUCTION



Thiophanate-methyl

On **April 8, 1999,** the Health Effects Division's Hazard Identification Assessment Review Committee evaluated the toxicology data base of thiophanate-methyl, reassessed the Reference Dose (RfD) established in 1986 and selected the toxicological endpoints for acute dietary as well as occupational/residential exposure risk assessments. The HIARC also addressed the potential enhanced sensitivity of infants and children from exposure to thiophanate-methyl as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in a report dated April 27, 1999 (HED Document No. 013360).

On **December 1999,** the HIARC reconsider the previous selection of the toxicity endpoint for inhalation exposure risk assessments; committee's conclusions are presented in the report dated December 16, 1999 (HED Document No 013947).

On **September 26, 2000,** the HIARC evaluated the results of a rabbit developmental toxicity study that was recently submitted to the Agency and its impact on FQPA assessment and the toxicity endpoint selection. This study conducted in 1997 was requested by the Agency to verify the equivocal findings seen in the 1986 rabbit developmental toxicity study. The Committee's conclusions from this meeting are presented in this report

II. HAZARD IDENTIFICATION

A.1. Acute Reference Dose (Acute RfD) Subpopulation Females 13 -50

Study Selected: Developmental toxicity in the rabbit **Guideline #:** 83-3(b)

MRID No.: 45051001

Executive Summary:

In a developmental toxicity study (MRID 45051001) thiophanate-methyl (97.28% purity) was administered to groups of 20 New Zealand White Rabbits by gavage in a 1% aqueous methyl cellulose vehicle (at a rate of 10 mL/kg) at dose levels of 0, 5, 10, 20 or 40 mg/kg/day on gestation days 6 to 28. The rabbits were sacrificed on day 29 and the does were subjected to uterine examination and the pups subjected to external, visceral and skeletal examination.

At 20 mg/kg/day there was **decreased body weight *gain*** (56%, < 0.05) for the interval days 12-15 and body weight gain was decreased 13% for the entire dosing period. At 40 mg/kg/day, body weight gain was decreased and there was actual body weight loss for the interval days 6-9 (i.e. the controls gained 80±40 g while the 40 mg/kg/day dose group actually lost 110±100 g). Final (day 29) body weight of the does in the high dose group was 6% less than the control. **Decreased food consumption** accompanied the decrease in body weight with there being 13 to 20% decrease in the 20 mg/kg/day dose group and 24 to 70% decreased in the high dose group. The high dose group also had more does with scant or no feces. There were no abortions. **The LOAEL for maternal toxicity is 20 mg/kg/day based on body weight and food consumption decreases. The NOAEL is 10 mg/kg/day.**

At 40 mg/kg/day, there were statistically significant ($p < 0.01$) *increases* in the mean number of ossification sites in the thoracic vertebrae (+3.12%) and ribs-pairs (+3.21%) as well as a *decrease* in lumbar vertebrae (-6%) and the differences were in excess of or less than the historical control range respectively. These conditions were collectively referred to as an *increase* in "**supernumerary ribs**" by the study author and were described as a reversible condition. There were also decreases (not statistically significant) in fetal weight (-9.6% for males and -6.6% for females). **The LOAEL is 40 mg/kg/day based on supernumerary ribs and decreased fetal weight The NOAEL is 20 mg/kg/day.**

Classification: This study is classified as ACCEPTABLE/GUIDELINE and satisfies the requirement for a series 83-3 developmental toxicity study in rabbits.

Dose and Endpoint Selected for Establishing the Acute RfD: Developmental NOAEL = 20 mg/kg/day based on supernumerary ribs at 40 mg/kg/day

Uncertainty Factor (UF): 100 (10 for inter-species extrapolation and 10 for intra-species variation).

$$\textbf{Acute RfD (Females 13+)} = \frac{\text{20 mg/kg/day (NOAEL)}}{\text{100 (UF)}} = \textbf{0.2 mg/kg}$$

Comments about Study/Endpoint/Uncertainty Factor(s): The developmental effects are presumed to occur after a single exposures. Since the effects occur in utero, its applicable only to the population subgroup (females of child bearing age).

A.2. Acute Reference Dose (Acute RfD) General Population (including infants and children)

Study selected: Chronic Oral Toxicity Study in the Dog **Guideline #:** 83-1(b)

MRID No.: 42311801

Executive Summary: In a chronic oral toxicity study (MRID 42311801), 4 beagle dogs/sex/dose group were administered thiophanate-methyl (tech., 96.55% a.i.) daily for 1 year by gelatin capsule at doses of 0, 8, 40 or 200 mg/kg/day.

At 40 mg/kg/day, decreased mean body weight/weight gain (compared to controls at termination, -7%/-19%, males and -6%/-19%, females; not statistically significant), decreased mean serum T4 levels in males at 6 months (-46%) and markedly increased TSH in 1 male at 6 and 12 months (approximately 2-fold over pretest), increased serum cholesterol in males at 6 and 12 months (+47% and +30%; latter not significant), increased absolute/relative thyroid weights (+33%/+42%, males and +28%/+10%, females; not statistically significant) and thyroid follicular epithelial cell hypertrophy (2/4 females) were observed.

At 200 mg/kg/day, tremors (mostly moderate in all dogs; observed 2-4 hrs post-dosing between days 1-17), slightly decreased Hgb, Hct and RBC in males at 6 and 12 months (-13% to -14% below controls), increased serum ALP at 6 and 12 months (+100% and +300%, males and +47% and +82%, females; not significant in females) and cholesterol at 6 and 12 months (+51% and +42%, males; latter not significant and +93% and +76%, females), increased relative liver weights (+46%, males and +35%, females) and thyroid follicular epithelial cell hyperplasia (1 male and 1 female) were observed. Decreases in body weight/weight gain, increases in thyroid weight and follicular cell hypertrophy and effects on thyroid hormones were more pronounced than at 40 mg/kg/day. Slight decreases in serum A/G ratio, Ca^{++}, K^{+} and phosphate in males were reported but not considered toxicologically significant. There were no treatment-related effects on survival, ophthalmologic parameters or urinalysis. **The LOAEL is 40 mg/kg/day, based on decreased body weight/weight gain and thyroid effects. The NOAEL is 8 mg/kg/day.**

This study is classified **Acceptable (§83-1b)** and satisfies the guideline requirement for a chronic oral toxicity study in the dog.

Dose and Endpoint Selected for Establishing the Acute RfD: 40 mg/kg/day, based on tremors seen 2-4 hours after dosing on 1-day at 200 mg/kg/day.

Uncertainty Factor (UF): 100 (10 for inter-species extrapolation and 10 for intra-species variation).

$$\textbf{Acute RfD (General Population)} = \frac{\text{40 mg/kg/day (NOAEL)}}{\text{100 (UF)}} = \textbf{0.4 mg/kg/day}$$

Comments about Study/Endpoint/Uncertainty Factor: This dose and endpoint are appropriate because although the study NOAEL for the full term of the study is 8 mg/kg/day, the HIARC selected the NOAEL of 40 mg/kg/day for establishing the acute RfD based on tremors seen 2-4 hours after dosing on Day 1 in all dogs at 200 mg/kg/day.

B. Chronic Reference Dose (Chronic RfD)

Study selected: Chronic oral toxicity study in dogs **Guideline #:** 83-1(b)

MRID No.: 42311801

Executive Summary: Refer to Acute Reference Dose, General Population, above

Dose and Endpoint Selected for Establishing the Chronic RfD: 8.0 mg/kg/day based on decreases in body weight/body weight gain and thyroid effects at 40 mg/kg/day

Uncertainty Factor (UF): 100 (10x for inter-species extrapolation and 10x for intra-species variability)

$$\textbf{Chronic RfD} = \frac{\text{8.0 mg/kg/day (NOAEL)}}{\text{100 (UF)}} = \textbf{0.08 mg/kg/day}$$

Comments about Study/Endpoint/Uncertainty Factor: At 40 mg/kg/day, decreased mean body weight/weight gain (compared to controls at termination, -7%/-19%, males and -6%/-19%, females; not statistically significant), decreased mean serum T4 levels in males at 6 months (-46%) and markedly increased TSH in 1 male at 6 and 12 months (approximately 2-fold over pretest), increased serum cholesterol in males at 6 and 12 months (+47% and +30%; latter not significant), increased absolute/relative thyroid weights (+33%/+42%, males and +28%/+10%, females; not statistically significant) and thyroid follicular epithelial cell hypertrophy (2/4 females) were observed.

The Agency previously established an RfD of 0.08, based on decreased body weight, decreased spermatogenesis and hyperthyroidism in a 2-year chronic toxicity feeding study in the rat (MRID 00117868). This value was established by HED after review on 2/21/86 and was verified by the Agency on 3/11/86 (note: this study has since been reclassified as Unacceptable based on numerous study deficiencies). The 1-year dog study and other acceptable chronic toxicity studies were submitted subsequent to establishment of this RfD.

C. Occupational / Residential Exposure

Thiophanate-methyl has registered residential uses (e.g., turf) that may result in post-application residential exposure. Therefore, endpoints were selected for incidental oral exposure to toddlers.

1. Short-Term Incidental Oral Ingestion (1 - 7 days)

Study Selected: Developmental toxicity in rabbits. **Guideline #:** 83-3(b)

MRID No.: 45051001
Executive Summary: Refer to Acute RfD -Subpopulation Females 13-50.

Dose and Endpoint Selected for Risk Assessment: NOAEL = 10 mg/kg/day based on decreases in body weight gain and food consumption at 20 mg/kg/day.

Comments about Study/Endpoint: The decreases in body weight gain and food consumption are considered appropriate endpoints for the population (toddlers) and duration (1-7 days) of concern.

2. Intermediate -Term Incidental Oral Ingestion (7 days to Several Months)

Study Selected: Developmental toxicity in rabbits. **Guideline #:** 83-3(b)

MRID No.: 45051001

Executive Summary: Refer to Acute RfD -Subpopulation Females 13-50.

Dose and Endpoint Selected for Risk Assessment: NOAEL = 10 mg/kg/day based on decreases in body weight gain and food consumption at 20 mg/kg/day.

Comments about Study/Endpoint: The decrease in body weight gain and food consumption are considered appropriate endpoints for the population (toddlers) and duration (7 days to several months) of concern.

3. Dermal Absorption

Comments about Dermal Absorption: No dermal absorption studies are available for thiophanate-methyl. A dermal absorption rate of 7% may be estimated based on the results of an oral developmental toxicity study and a 21-day dermal toxicity study in the same species (rabbit) with similar endpoints (decreased food consumption).

In the 1997 oral developmental toxicity study in the rabbit, the maternal toxicity LOAEL was 20 mg/kg/day, based on decreased food consumption and body weight gain (MRID No.45051001).

In the 21-day dermal toxicity study in the rabbit, the systemic toxicity LOAEL was 300 mg/kg/day, based on decreased food consumption in females (MRID 42110801).

A ratio of the LOAELs from the oral and dermal studies indicated a dermal absorption rate of about 7% (oral LOAEL of 20 mg/kg/day ÷ dermal LOAEL of 300 mg/kg/day $\approx$7%).

Benomyl, a related carbamate fungicide compound with a common metabolite to thiophanate-methyl (MBC), shows absorption up to 3.5%.

The molecular weight of thiophanate-methyl is 342.4 g. Solubility in water is 22 ppm at 25°C and it is sparingly soluble in most organic solvents. The structure is shown above in the Introduction of this document.

4. Short-Term Dermal (1 - 7 days)

Study Selected: 21-day dermal toxicity study in rabbits. **Guideline #:** 83-3(b)

MRID No.: 42110801

Executive Summary: (prepared for this HIARC report by J. Doherty from information in DER prepared by W. Greear dated July 15, 1992 and from the revised Executive Summary prepared by Linnea Hansen dated March 22, 1999.

In a 21-day dermal toxicity study (MRID No.: 42110801), five New Zealand White rabbits/sex/dose group were exposed dermally to thiophanate-methyl (technical 96.55%) for six hours/day, five days per week over a period of 21 days (total of 15 exposures). The skin was shaved prior to treatment. The test material was moistened with distilled water prior to application and spread over the treated area.

Mild dermal irritation was noted at 100 mg/kg/day and higher. At 300 mg/kg/day, **food consumption** in females was statistically significantly decreased during weeks 1 and 3 (18% and 15% $p < 0.05$). Commutative consumption was decreased 9.1% for males and 15% for females (not statistically significant) At 1000 mg/kg/day, commutative food consumption was decreased 20% for both males and females. The **body weight gains** for females were 8%, 28% and 30% decreased for the low, mid and high dose groups. Male body weight gain did not show a consistent decrease. **The systemic LOAEL is 300 mg/kg/day based on decreased food consumption and probable body weight decrease in females. The NOAEL is 100 mg/kg/day.**

This study is classified as ACCEPTABLE/Guideline and satisfies the requirement for a 21-day dermal toxicity study in the rabbit.

Dose and Endpoint Selected for Risk Assessment: NOAEL = 100 mg/kg/day based on dose-dependent and statistically significant decreases in food consumption in females at 300 mg/kg/day.

Comments about Study/Endpoint: Although no other signs of systemic toxicity were observed, the dose-dependent decreased food consumption in females at mid (300 mg/kg/day) and high (1000 mg/kg/day) doses with a probable associated decrease in body weight gain and decreased consumption in males at the mid (9%) and high (20%) dose although not statistically significant suggests that the test material was absorbed. The dose (100 mg/kg/day) is protective of the developmental effects since 1) maternal toxicity (LOAEL, 20 m/kg/day) occurred at a lower dose than developmental toxicity (LOAEL, 40 mg/kg/day), and 2) the maternal effects (decreases in food consumption) were also seen following dermal exposure in the same species (rabbits). Additionally, the route (dermal) and duration of treatment (21-days) is appropriate for this exposure scenario.

5. Intermediate-Term Dermal (7 days to Several Months)

Study Selected: 21-day dermal toxicity study in rabbits. **Guideline #:** 83-3(b)

MRID No.: 42110801

Executive Summary: See Short-Term

Dose and Endpoint Selected for Risk Assessment: NOAEL = 100 mg/kg/day based on dose-dependent and statistically significant decreases in food consumption in females at 300 mg/kg/day.

Comments about Study/Endpoint: The HIARC determined that this study is appropriate for use for this exposure scenario since the dose (100 mg/kg/day) used for this risk assessment is comparable to the dermal equivalent dose (114 mg/kg/day) derived by using the oral NOAEL of 8 mg/kg/day and 7% dermal absorption factor (8 mg/kg/day ÷ 0.07 = 114 mg/kg/day) used for Long-Term exposure scenario. Additionally, use of the 21-day dermal study minimizes the uncertainties of route to route extrapolation and the use of an uncertain dermal penetration factor.

6. Long-term Dermal (Several Months to Lifetime)

Study Selected: Chronic oral toxicity study in dogs **Guideline #:** 83-3(b)

MRID No.: 42311801

Executive Summary: Refer to Chronic RfD.

Dose Selected for Risk Assessment: NOAEL = 8 mg/kg/day based on decreases in body weight/body weight gain and thyroid effects at 40 mg/kg/day.

Comments about Study/Endpoint: The HIARC noted that the NOAEL of 100 mg/kg/day established in the 21-day dermal study is comparable to the dermal equivalent dose of 114 mg/kg/day derived by the using the oral NOAEL of 8 mg/kg/day and 7% dermal absorption (8 ÷ 0.07= 114). However, the 21-day dermal study was not selected for this exposure scenario because of the concern for the thyroid effects seen after chronic exposure in the dog study. This study/dose was also used for establishing the Chronic RfD. Since an oral NOAEL was selected a 7% dermal absorption factor must be used for route-to-route extrapolation.

7. Inhalation Exposure (Any Time Period)

Thiophanate-methyl is a category III acute inhalation toxicant (LC_{50} = 1.7 mg/L, males and 1.9 mg/L, females). Inhalation (occupational) exposure is anticipated based on the current use patterns and therefore this risk assessment is required. On December 1999, the HIARC determined that the 14-day inhalation toxicity study is not appropriate for use in assessing the inhalation exposure risk because aside from the fact that the study tested a

formulation product, this study did not evaluate all of the standard parameters (e.g., clinical chemistry, hematology, organ weights, complete gross/microscopic tissue evaluation) and therefore does not provide a complete profile of inhalation toxicity. This study is classified as unacceptable, and is not appropriate for risk assessment.

Therefore, the HIARC selected oral NOAELs for inhalation exposure risk assessments with the route-to-route extrapolation as indicated below:

Convert the inhalation exposure component (i.e., µg/kg/day a.i/day) using a 100% absorption rate (default value) and the application rate to an **oral equivalent dose** (mg/kg/day)

Compare the oral equivalent dose to the oral NOAELs to calculate the Margins of Exposure.

Short-Term: Oral (maternal) NOAEL = 10 mg/kg/day - Developmental Toxicity study - Rabbit

Intermediate-Term: Oral (maternal) NOAEL = 10 mg/kg/day - Developmental Toxicity study- Rabbit

Long-Term: Oral NOAEL of 8 mg/kg/day - Chronic Toxicity Study in Dogs

D. Margins of Exposure for Occupational/Residential Exposures

A MOE of 100 is adequate for dermal and inhalation risk assessments. The MOEs for residential exposure risk assessments will be determined by the FQPA Safety Factor Committee.

E. Recommendation for Aggregate (Food + Water + Residential) Risk Assessments

Thiophanate-methyl has registered residential uses (e.g., turf) that may result in post-application residential exposure, as well as food uses. Its mobility in soil is moderate and therefore may enter into ground water. Therefore, aggregate exposure risk assessment will be for combined food + water + residential exposures.

For acute aggregate exposure risk assessment, combine high-end exposure values from food and water and compare to the acute RfDs adjusted for the FQPA factor (i.e., the Population Adjusted Dose, PAD).

For short and intermediate-term aggregate exposure risk assessment, the oral component can be combined with dermal and inhalation because of a common toxicological endpoint (decreases in food consumption) seen via the oral (during gestation days 12-15), dermal (following dermal exposure) and inhalation (oral equivalent) routes.

For long-term aggregate exposure risk assessment, exposures from oral, dermal and inhalation can be combined since dermal and inhalation exposures are corrected to oral equivalent doses.

III. CARCINOGENICITY SCREEN:

A. Combined Chronic Toxicity/Carcinogenicity Study in Rats

Executive Summary: In a 2-year feeding/oncogenicity study (MRID 42896601), thiophanate methyl was administered in the diet to 60 male and 60 female F344 rats/group at 0, 75, 200, 1200 or 6000 ppm. After week 52, 10 rats/sex/dose were sacrificed, except only five 6000 ppm males were sacrificed because 8 males died from non-treatment related injury at weeks 11 and 12. The mean compound consumption for the study was 0, 3.3, 8.8, 54.4 and 280.6 mg/kg/day for males and 0, 3.8, 10.2, 63.5 and 334.7 mg/kg/day for females.
Rats fed 75 and 200 ppm had no significant treatment-related toxic effects. Male rats fed 1200 ppm and 6000 ppm had significantly decreased mean body weights and net weight gains at the end of the study. The mean weight of 1200 ppm males was 84% of controls ($p<0.001$), and the net gain was 79% of controls ($p<0.001$), whereas the two 6000 ppm males which survived to week 104 had a mean weight 73% of controls and net weight gain 63% of controls. Female rats had significant body weight changes only in the 6000 ppm dose group, the mean weight was 78% ($p<0.001$) and the mean net gain was 69% ($p<0.001$) of controls at the end of the study. The 1200 and 6000 ppm males and females had decreased food efficiency. Food efficiency in rats fed 1200 ppm was reduced to 78% and 88% of controls in males and females, respectively, while in 6000 ppm rats, the efficiencies were lowered to 65% and 71% in males and females. There was a treatment-related decrease in survival in only the 6000 ppm group males (2/55 survivors vs. 37/50 controls, $p<0.001$); the marginal increase in mortality ($p<0.05$) in the 200 ppm group males appeared spurious. Other male groups and all female dose groups were unaffected. Non-neoplastic pathological changes were observed primarily in 1200 and 6000 ppm rats in the liver (dose-related weight increase and hepatocellular hypertrophy), kidney (surface changes, dose-related increase in weight and severity of nephropathy), and thyroid (dose-related weight increase, follicular cell hypertrophy and hyperplasia, and T_3 and T_4 hormone level decreases). The levels of thyroid stimulating hormone (TSH) were elevated, though pituitary weights were unchanged. **A LOAEL of 1200 ppm was identified for both male (54.4 mg/kg/day) and female (63.5 mg/kg/day) rats, based on treatment-related effects in the liver, kidneys, and thyroid and decreased body weight in males. The corresponding NOAEL was 200 ppm in both sexes of rats (corresponding to 8.8 mg/kg/day for males and 10.2 mg/kg/day for females), based on lack of significant toxic effects at this dose**.

The toxic effects observed in the thyroid in 1200 and 6000 ppm male and female rats were accompanied by a dose-related increase in the incidence of follicular cell adenoma (males: 1/50, 0/48, 0/50, 3/50, 12/55 and females: 0/50, 0/49, 0/50, 1/50, 2/50 for doses of 0, 75, 200, 1200, and 6000 ppm, respectively). The increase was statistically significant ($p<0.01$) only in males at 6000 ppm, a dose which was shown to exceed the maximum tolerated by males by the high mortality it caused. The thyroid adenoma was likely a secondary effect of the thyroid-pituitary hormonal imbalance induced by chronic compound treatment. The increased incidences of neoplasms in the spleen and adrenal medulla were not dose-related and were of uncertain biological significance (spleen mononuclear cell leukemia in 75 and 200 ppm males and in 75, 200, and 1200 ppm females and adrenal medulla pheochromocytoma in 75, 200, and 1200 ppm

males). There were also several neoplasms which were statistically elevated but incidental to treatment (skin papilloma in 75 ppm males, pituitary adenoma in 200 ppm males and mammary gland fibroadenoma in 1200 ppm females). Based on the significant depressions in mean body weights and mean net body weight gains in the rats, it appears that the maximum tolerated dose (MTD) was achieved in the study for both males (1200 ppm or 54.4 mg/kg/day) and females (6000 ppm or 334.7 mg/kg/day).

MRID No.: 42896601

Discussion of Tumor Data: At 6000 ppm, the incidence of thyroid follicular cell adenoma was increased in males (from control to high dose, 2%, 0%, 0%, 6%, 24%) and females (from control to high dose, 0%, 0%, 0%, 2% and 6%). The increase was statistically significant only in males at 6000 ppm ($p<0.01$), which was considered an excessive dose (see below). Other tumors were observed at increased incidence above controls but did not show a dose-response and were not considered treatment-related. These included (1) increased incidence of adrenal medulla pheochromocytoma at 75, 200 and 1200 ppm in males and (2) increased incidence of mononuclear cell leukemia in the spleen in males at 75 and 200 ppm in males and 75, 200 and 1200 ppm in females (incidence of this leukemia in specific organs does not reflect total incidence within a group).

Adequacy of the Dose Levels Tested: In males at the highest dose tested (6000 ppm), dosing was considered excessive and the MTD was exceeded based on excessive mortality. Dosing was considered adequate at 1200 ppm based on thyroid, liver and kidney effects in both sexes and decreased body weight/weight gain in males; dosing in females at 5000 ppm did not exceed the MTD.

B. Carcinogenicity Study in Mice

Executive Summary: In a dietary carcinogenicity study (MRID 42607701), thiophanate-methyl (tech., 95.93 to 96.55% a.i.) was administered daily to 50 CD-1 mice/sex/dose at concentrations of 0, 150, 640, 3000 or 7000 ppm for 18 months (equivalent to average daily intakes of 0, 23.7, 98.6, 467.6 or 1078.8 mg/kg/day, males and 0, 28.7, 123.3, 557.9 or 1329.4 mg/kg/day, females). An additional 10 mice/sex/dose were administered these dose levels and sacrificed at 39 weeks.

At 640 ppm, increased incidence of hepatocellular hypertrophy was observed in females (8% vs. 0% affected, controls). At 3000 ppm, slightly decreased mean body weights in males, primarily during the middle of the study (<8% below controls; gain -12% below controls at week 53), transiently increased TSH (week 39, +100% above controls), increased abs/rel thyroid weights in males (+52%/+64% above controls, week 39 only), increased abs/rel liver weights (+20 to +26% above controls, males and females), increased incidence of hepatocellular hypertrophy in males and females (25% affected vs. 10%, controls and 10% vs. 0%, controls, respectively) and increased incidence of atrial thrombosis in females (35% vs. 0%, controls) were observed. At 7000 ppm, decreased survival (males 52% vs. 82%, controls; females 54% vs. 76%, controls), decreased mean body weight and weight gain, primarily during the middle of the study (body weight -3% to -8% less than controls and gain at 53 weeks -12%, females and -16%, males); decreased mean body weight at termination in females (-8%) but not males; slightly decreased RBC count in males (-15%); decreased T4 in females (-28%, week 39); increased abs/rel liver

weights (at wks 39 and 79, males +34%/+40% and +82%/+86%; females +57%/57% and +31%/31%), abs/rel thyroid weights (at wk 39, males >2-fold increase; females +30%) and abs/rel heart weights in females (+23%/+40%, wks 39 and 79); and increased incidence of hepatocellular hypertrophy in males and females (42% and 20% affected) and atrial thrombosis in males (16% vs. 2%, controls) and females (28%) were observed. **The systemic toxicity LOAEL is 640 ppm (123.3 mg/kg/day), based on hepatocellular hypertrophy in females. The NOAEL is 150 ppm (28.7 mg/kg/day). (The systemic toxicity LOAEL in males is 3000 ppm or 467.6 mg/kg/day, based on decreased body weight/weight gain, increased thyroid and liver weights and hepatocellular hypertrophy. The NOAEL is 640 ppm or 98.6 mg/kg/day).**

Hepatocellular adenoma showed statistically significant, dose-related increases in both sexes at 3000 and 7000 ppm (from control to high dose, 7%, 13%, 12%, 32% and 40%, males and 0%, 0%, 5%, 13% and 30%, females; all animals on study).

Dosing was considered adequate in both sexes based on body weight/weight gain decreases and increased thyroid weights in males, liver effects in both sexes and atrial thrombosis in females at 3000 ppm, as well as additional effects at 7000 ppm.

This study is classified **Acceptable (§83-2b)** and satisfies the guideline requirement for a carcinogenicity study in the mouse.

MRID No.: 42607701

Discussion of Tumor Data: The incidence of hepatocellular adenoma was increased (statistically significant, $p<0.01$) in both males and females at 3000 and 7000 ppm, the highest two dose levels tested. From control to high dose, the incidence was 7%, 13%, 12%, 32% and 40% in males and 0%, 0%, 5%, 13% and 30% in females.

Adequacy of the Dose Levels Tested: Dosing was considered adequate in this study based on decreased body weight/weight gain and increased thyroid weights in males, liver effects in both sexes and atrial thrombosis in females at 3000 ppm, as well as additional effects at 7000 ppm

C. Classification of carcinogenic potential

In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996), the Committee classified thiophanate-methyl as **"likely to be carcinogenic to humans"** by the oral route based on the following weight-of-the-evidence considerations:

Tumors were seen in both sexes of two species. Liver tumors were seen in male and female mice and thyroid tumors were seen in male and female rats. A dose-response in tumor incidence and progressive development of lesions were evident. Moreover, the same spectrum of effects was seen for both sexes in each species.

The relevance of the observed tumors to human exposure cannot be discounted

Thiophanate-methyl appears to be aneugenic. Methyl-2-benzimidazole carbamate (MBC), a metabolite of thiophanate-methyl, is a known aneugen, hepatocellular carcinogen and teratogen. MBC is also a metabolite of benomyl, an aneugen, which also produces liver tumors in mice. The potential for a direct DNA reactive mutagenic potential can not be dismissed by the available data.

There was some evidence of interference with thyroid pituitary homeostasis, an effect seen with other thiourea compounds. However, reversibility of the hormonal effect was not demonstrated, the studies were conducted only at an excessive dose and not at lower dose levels to demonstrate a dose-response. Additional studies are required to evaluate the direct mutagenic potential of thiophanate-methyl.

For human risk characterization, the extrapolation of risk using the linear low-dose (Q_1*) default approach for liver tumors was recommended. This extrapolation was supported by the lack of confirmation of the mode of action, concern for mutagenicity and dose-dependent increases in the incidence of liver tumors in male and female mice.

IV. MUTAGENICITY

The only acceptable genetic toxicology studies on thiophanate-methyl indicate that the compound is not clastogenic *in vitro* and did not cause unscheduled DNA synthesis in cultured rat hepatocytes. However, there are no acceptable gene mutation or *in vivo* assays. In contrast to the negative findings from the acceptable *in vitro* studies, data from the open literature show that thiophanate-methyl is positive for the induction of micronuclei but not structural chromosome aberrations in whole animals and caused cell transformation *in vitro.* The positive results from the *in vivo* micronucleus assay are consistent with the data from the common metabolite of benomyl and thiophanate-methyl (methyl-2-benzimidazolecarbamate, MBC) and benomyl indicating that both compounds are confirmed inducers of aneuploidy (adverse effects on chromosome numbers). Since aneuploidy may be involved in carcinogenesis, the weight-of-the-evidence from the genetic toxicology studies with thiophanate-methyl in conjunction with the findings for benomyl and MBC support the involvement of a genetic component in the data from 18-month chronic feeding study demonstrating hepatocellular carcinomas in male and female mice (MRID No.42607701). No correlation can be made relative to the possible role of aneuploidy as a contributing factor to birth defects since the rat developmental studies were considered unacceptable (MRID Nos. 00106090, 00146643/92186011); however, there was no indication of a developmental effect in these studies.

The acceptable studies do not satisfy either pre-1991 or new mutagenicity guideline requirements. In consideration of the results from the open literature, however, it is concluded that the acceptable studies submitted to the Agency combined with the data from the open literature studies satisfy the pre-1991 mutagenicity test guidelines, and that no further testing is warranted. Summaries of the acceptable mutagenicity studies and studies from the open literature are presented below:

SUBMITTED STUDIES

CHROMOSOME ABERRATIONS

1) *In vitro* mammalian cell cytogenetic assay in Chinese hamster ovary cells: The test is negative up to insoluble and cytotoxic doses (400 μg/mL -S9; $\geq$750 μg/mL +S9). Marked increases in mitotic delay were seen at >100 μg/mL -S9; >335 μg/mL +S9. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data (MRID No. 40980101).

OTHER MUTAGENIC MECHANISMS

2) *In vitro* unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes: The test is negative up to a cytotoxic and insoluble level (1000 μg/mL). The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a UDS assay (MRID No. 40095503).

OTHER INFORMATION

GENE MUTATIONS

In preincubation *Salmonella typhimurium* mammalian microsome gene mutation assays (Zeiger et al., 1992)[1], thiophanate-methyl ester (95.1%) produced weak equivocal responses (i.e., dose-related increases in revertant colonies of strains TA98 and TA100, which approximated $\geq$2-fold , at precipitating concentrations $\geq$3333.0 μg/plate in the presence of 30% hamster or rat S9 activation in one trial and negative results in a subsequent trial.

CHROMOSOME ABERRATIONS (SOMATIC CELLS)

Cytogenetic analysis performed by Barale et al. (1993)[2] showed that thiophanate-methyl significantly ($p<0.0001$) increased the frequency of micronuclei in the bone marrow cells of male Swiss albino mice 24 hours after receiving a single oral gavage dose of 1 gm/kg. No increase in structural chromosome aberrations was seen but a borderline significant increase in polyploidy and hyperploidy cells was detected. The data further indicate that thiophanate-methyl was less effective than benomyl or the common metabolite of both benomyl and thiophanate-methyl, (methyl-2-benzimidazolecarbamate, MBC), in the induction of micronuclei. In this study, MBC induced micronuclei in PCE more efficiently that either parent with benomyl induction followed by thiophanate-methyl induction. The combined results of this series of tests (i.e., positive for micronuclei induction/negative for structural chromosome aberrations) suggest that thiophanate-methyl probably interferes with the mitotic spindle rather than causing structural chromosomal damage and is, therefore, aneugenic. This conclusion is supported by the positive findings of an *in vivo* bone marrow micronucleus assay (MRID No. 41051510) conducted with benomyl and MBC and the positive *in vivo* bone marrow erythrocyte immunofluorescent antikinetochore antibody assays for detection of aneuploidy induction with benomyl (MRID No. 42911601) and MBC (MRID No. 42911602).

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[1]Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1992). Salmonella mutagenicity tests: IV Results of testing 311 chemicals. Environ and Mol Mutagen 19 [Suppl 21]:2-141.

[2]Barale, R., Scapoli, C., Meli, C., Casini, D., Minunni, M., Marrazzini, A., Loprieno, N. and Barrai, I. (1993). Cytogenetic effects of benzimidazoles in mouse bone marrow. Mutat Res. 300:15-28.

OTHER MUTAGENIC MECHANISMS–CELL TRANSFORMATION

In independently performed BALB/c3T3 *in vitro* cell transformation assays (Perocco et al., 1997)[3], thiophanate-methyl induced a significant ($p<0.001$) and reproducible increase in morphologically transformed foci in the absence of S9 activation at 25 μg/mL and significant ($p\leq0.001$) and reproducible dose-related increases in transformed foci at 20, 100 or 200 μg/mL in the presence of S9 activation.

CONCLUSIONS: Overall, the data from the submitted studies and the open literature indicate that thiophanate-methyl is neither clastogenic nor causes UDS in cultured mammalian cells. There is, however, reproducible positive results from *in vitro* cell transformation studies as well as micronuclei induction in the absence of structural chromosome aberrations *in vivo*. There is also convincing and supporting evidence that while both benomyl and the MBC metabolite induce micronuclei *in vivo*, neither compound is clastogenic. Hence, the available data for thiophanate-methyl are consistent with the genetic toxicology profiles for benomyl and MBC and indicate that thiophanate-methyl causes aneuploidy. Since it is generally acknowledged that somatic cell aneuploidy may be involved in carcinogenesis and the test article caused morphologically transformed cells *in vitro*, it is not surprising that the results from genetic toxicology testing with thiophanate-methyl correlate favorably with the data from the chronic feeding study demonstrating hepatocellular carcinomas in male and female mice (MRID No. 42607701). These data are also consistent with the results of the chronic mouse bioassay indicating that benomyl is a liver carcinogen (MRID No. 00096514). As an aneugen, the possible role of thiophanate-methyl in contributing to birth defects can not be determined at this time since both rat developmental studies were considered unacceptable. There was, however, no indication of a developmental effect in these studies.

In light of the evidence indicating that thiophanate-methyl is an aneugen, it is concluded that no additional genetic toxicology testing is warranted. The acceptable studies combined with the open literature studies satisfy the Pre-1991 mutagenicity initial testing battery guidelines and provide adequate data to draw meaningful conclusions.

STRUCTURAL ACTIVITY RELATIONSHIP (SAR)

Thiophanate-methyl is a member of the benzimidazole class of compounds which includes the fungicide pesticide benomyl, a known aneugen, hepatocellular carcinogen and teratogen. Both methyl-thiophanate and benomyl can convert to methyl-2-benzimidazolecarbamate (MBC), the active form. However, conversion of methyl-thiophanate to MBC proceeds at a slower rate than benomyl (Selling et al., 1970)[4]. Thus, the slower breakdown of thiophanate-methyl to MBC, compared to benomyl, may explain the less efficient production of micronucleated polychromatic

[3]Perocco, P., Del Ciello, C., Mazzullo, M., Rocchi, P., Ferreri, A.M., Paolini, M. Pozzetti, L. and Cantelli-Forti, G. (1997). Cytotoxicity and cell transforming activities of the fungicide methyl thiophanate on BALB/c 3T3 cells in vitro. Mutat. Res. 394:29-35.

[4]Selling, H.A., Vonk, J.W., Kaars Sijpesteijn, A. (1970). Transformation of the systemic fungicide methyl thiophanate in to 2-benzimidazole carbamic acid methyl ester, in: Chemistry and Industry, Madley, London, pp.1625-1626.

erythrocytes (PCEs) observed by Barale et al. (1993) [5] with thiophanate-methyl.

V. FQPA CONSIDERATIONS

A. Adequacy of the Data Base

Acute and subchronic neurotoxicity screening studies in the rat have not been submitted. The HIARC re-affirmed the previous decision and requested submission of these studies because tremors were observed in the chronic dog study. The database is otherwise adequate to assess the potential susceptibility to infants and children. This includes prenatal developmental toxicity studies in rats via the gavage and dietary administration, two prenatal developmental toxicity studies in rabbits and two reproduction toxicity studies.

A delayed neurotoxicity study in the hen is not required because thiophanate-methyl is not an organophosphate.

B. Neurotoxicity Data

Neurotoxicity Studies: Acute and 90-day rat neurotoxicity screening studies with thiophanate methyl have not been submitted. A hen acute delayed neurotoxicity study (MRID 00081603) was submitted but was classified as unacceptable (no delayed neurotoxic effects were reported). This study is not required for thiophanate-methyl because it is not an organophosphate.

Evidence of Neurotoxicity From Other Oral Toxicity Studies: A potential indication of neurotoxicity in the current database was the occurrence of tremors in the high dose group in the 1-year oral toxicity study in beagle dogs (MRID 42311801). Tremors were observed at the daily cageside evaluations within 2-4 hrs after dosing as follows: moderate in 7/8 high dose animals on day 1, slight in 3/4 high dose males on days 7, 12 or 13 and severe tonic convulsions in 1/4 high dose female on days 2, 16 and 17. No tremors were reported after day 17.

SAR Considerations: Thiophanate-methyl and benomyl share a common metabolite, MBC. In the rat acute neurotoxicity screening study on benomyl (MRID 42817003), slightly decreased (-6%) brain weight in males and in the rat subchronic neurotoxicity screening study (MRID 43277901), increased motor activity in females were considered potential indications of neurotoxicity (12/3/97 HIARC memorandum). CNS and ocular effects were also reported in rat developmental toxicity studies on benomyl (MRIDs 00148393, 00119017; also in published reports) and in the rabbit developmental toxicity study on MBC (Accession No. 260571).

C. Developmental & Reproductive Toxicity

(1) Developmental Toxicity:

Rat - Two developmental toxicity studies were available in this species; in one study, thiophanate-methyl was administered via gavage and in the other via dietary administration.

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[5]Barale, R. et al., . Mutat Res. 300 (1993):15-28.

In the **gavage study,** (MRID 00106090), 25 presumed pregnant Charles River COBS® CD® rats per group were administered Thiophanate-methyl (97.2% a.i.; Lot No. TM-123) daily by **gavage** at doses of 0, 100, 300 or 1000 mg/kg/day on gestation days (GD) 6-19, inclusive. On GD 20, dams were sacrificed and subjected to gross necropsy. At necropsy, the fetuses were weighed, sexed and examined for external malformations and variations, including the palate and eyes. Approximately one-half of the fetuses were placed in Bouin's fixative for subsequent soft tissue examination. The remaining one-half of the fetuses were fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S for subsequent skeletal examination.

At 1000 mg/kg/day, a statistically significant reduction in body weight gain during the first 3 days of treatment (-100% of controls from GD 6-9, $p<0.05$) was observed. Reduced body weight gain (not statistically significant) during the dosing interval of GD 9-12 (-17%), and for the entire dosing period (-10%; measured from GD 6-20) was observed. Mean absolute body weights were not significantly affected by treatment. At 100 and 300 mg/kg/day, body weight gain was statistically significantly reduced ($p<0.05$) during the first three days of treatment as compared to controls (-56% of controls for both the 100 and 300 mg/kg/day groups); however, body weight gain in these groups was comparable to controls for the remainder of dosing and over the entire dosing period (measured between GD 6-20). No treatment-related gross pathological changes were noted. Individual or summary data of maternal antemortem/daily observations were not included: the study author stated in the text that no treatment-related clinical signs of toxicity were noted. **The maternal toxicity LOAEL is 1000 mg/kg/day based on reduced body weight gain. The maternal toxicity NOAEL is 300 mg/kg/day.** This LOAEL/NOAEL is contingent upon individual/summary data of clinical signs not revealing any significant treatment-related effects at lower doses, and upon test substance analyses verifying that test material stability, homogeneity, and concentration of the dosing medium are acceptable.

No developmental effects were reported in the data provided by the study author in the summary tables at any dose tested; however, incidence rates or calculation of overall litter incidences for malformations/variations could not be conducted because individual fetal data were not provided. **Therefore, a tentative developmental toxicity LOAEL is >1000 mg/kg/day and NOAEL is ≥1000 mg/kg/day.**

This study is classified as **Unacceptable (§83-3(a))** (**upgradable**) and does not satisfy the Subdivision F requirements for a developmental toxicity study in rats because analyses for test material stability, homogeneity, and concentration in dosing medium were not provided, individual or summary data of maternal antemortem/daily observations were not available, food consumption data were not provided and individual fetal examination data for external, visceral, and skeletal variations were not provided. This study may be **upgradable** if the missing data are supplied. The reduced number of litters in most groups (18, 19, 17 and 16 for the 0, 100, 300 and 1000 mg/kg/day groups, respectively; guideline minimum is 20 litters/group) and the lack of adjustment for dose concentrations with increases in body weights are not considered to have significantly compromised the study conclusions. The limit dose was tested and the pilot study testing doses of up to 5000 mg/kg/day resulted only in moderate maternal body weight decreases.

In 1985, HED reevaluated another developmental toxicity study in rats by the dietary route of administration. This study was requested since the results of the 2-generation

reproduction study (MRID No.:42899101 to -05 and 43624401) indicated that dietary administration is the preferred route of exposure for assessing thiophanate methyl for teratogenic potential. (Memorandum from R. Gardner to P. Hundemann dated May 21, 1985, HED Document No.: 004459).

In the **dietary** administration study, (MRID 00146643), 25 presumed pregnant Crl:COBS® CD® (SD) BR strain rats per group were fed Thiophanate-methyl (95.3% a.i.) at dietary concentrations of 0, 250, 1200 or 2500 ppm (equivalent to 0, 18, 85 or 163 mg/kg/day) on gestation days (GD) 6 through 19, inclusive. On GD 20, dams were sacrificed and subjected to gross necropsy. At necropsy, the gravid uteri and individual fetuses from each dam were weighed, and the numbers of corpora lutea, implantation sites, live and dead fetuses, and embryonic deaths were noted. Crown-to-rump length was measured for all fetuses. All fetuses were sexed and examined for external malformations and variations.

Maternal Toxicity: Decreased food consumption and associated body weight effects were noted at 1200 and 2500 ppm levels. The reduced food consumption was significant during days 6-12 for the1200 ppm group (13-18% reduction) and days 6-15 for 2500 ppm group (17 to 40% reduction) when food consumption was decreased as much as 40% just after dosing began (days 6-9 for 2500 ppm group). The decreased food consumption in the 1200 and 2500 ppm groups was accompanied by decreases in net body weight (2% and 5.6% decreases relative to controls without gravid uterus, respectively), body weight gain (25% and 36% decreases relative to controls, respectively), and uterine weight (3.4% and 11.3%, respectively) but the effects were reversible. It is possible that the decreased food consumption was due to decreased palatability of the test diets. Alopecia was the most frequently reported clinical sign that was observed in 3-6 animals in all groups and was noted prior to study initiation. The frequency and number of animals affected by alopecia was slightly higher in the high dose group. There were no significant differences reported between groups for number of corpora lutea, implantations, resorptions (early and late), or live fetuses per dam. **The maternal toxicity NOAEL is 250 ppm (18 mg/kg/day) and the maternal toxicity LOAEL is 1200 ppm (85 mg/kg/day) based on significantly reduced food consumption.**

Developmental Toxicity: There were no treatment-related effects on fetal weight, crown to rump length, external variations or fetal malformations. The most frequently reported soft tissue observation was undeveloped renal papillae and/or distended ureters that occurred in 10, 4.3, 25 and 13% of the litters, of the 0, 250, 1200 and 2500 ppm groups, respectively. These observations were not considered treatment-related because they were not dose-related. The most frequently reported skeletal variations were unossified sternebrae (#5 and #6) that occurred in 55, 52.2, 66.7 and 65.4% of the litters (12.4, 17.4, 17.3 and 15% of fetuses), respectively, and 14^{th} rudimentary ribs that occurred in 45, 47.8, 41.7 and 39.1% of the litters (5.4, 5.4, 4.4 and 3.7% of fetuses), respectively. However, the incidence of unossified sternebrae in fetuses and litters was not statistically significant, and was within the reported historical control range. **The developmental toxicity NOAEL is 2500 ppm (163 mg/kg/day) (highest dose tested) based on an absence of treatment-related effects in the fetuses.**

This study is classified as **Acceptable-Guideline**, and does satisfy the §83-3 (a) Subdivision F requirements for a developmental toxicity study in rats.

The gavage study in which no maternal nor developmental toxicity were seen at the Limit Dose was originally classified as Unacceptable but upgradable pending receipt of additional information (e.g., analysis of the dosing solution, individual animal data) requested by HED. In light of the results of the gavage study, the Agency requested a dietary administration study to evaluate the teratogenic potential of thiophanate methyl when administered in the diet rather than gavage.

The dietary study also showed no developmental toxicity at the highest dose tested (2500 ppm or 163 mg/kg/day). The results of the two studies are consistent with each other and the two studies together establishing NOAEL for maternal toxicity based on reduced food consumption. In addition, a pilot gavage study at doses up to 5000 mg/kg/day only moderate maternal body weight decrements were seen. The TOX SAC recommended (refer to TOX SAC report from Joycelyn E. Stewart dated March 23, 2000) that the dietary developmental toxicity study in rats be classified as ACCEPTABLE-Nonguideline and that the study satisfies the requirement for a Subdivision F guideline study for developmental tonicity in the rat. Consequently, it is not surprising that only minimal toxicity was seen via the dietary study. In summary, the results of these two studies together indicate that thiophanate methyl is not a developmental toxicant in rats.

Rabbit - Two developmental toxicity studies were available in this species. In a study conducted in 1986 in a testing facility in England and the other at a testing facility in the U.S.A

First Study (1986).

Executive Summary: The following Executive Summary has been revised by J. Doherty as per the outcome of this HIARC meeting.

In a developmental toxicity study (MRID 40022801), 15 pregnant (artificially inseminated) New Zealand white rabbits/dose group were administered thiophanate-methyl (tech., 96.2% a.i.) by gavage at doses of 0, 2, 6 or 20 mg/kg/day from days 6 through 19 of gestation. Test material was administered in 1% w/v aqueous methyl cellulose.

Maternal Toxicity: At 20 mg/kg/day, decreased mean body weight (transient, maximum -8.6% below controls at day 10 due to weight loss during days 6-10 of gestation), decreased food consumption (transient, -38% between days 6-12 of gestation), decreased fecal output and increased incidence of abortion/total litter loss (2 abortions/1 total litter loss vs. 0 abortions/1 total litter loss, controls) were observed. No clinical signs or gross findings were observed. **The maternal toxicity LOAEL is 20 mg/kg/day, based on increased incidence of abortions and decreases in body weight and food consumption. The NOAEL is 6 mg/kg/day.**

Developmental Toxicity: An issue of a possible increase in "asymmetric pelvis" was indicated by there being an apparent increased incidence (not statistically significant, fetal incidence 7.4% vs.

3.4%, controls; litter incidence 42% vs. 25%, controls) at 6 mg/kg/day. At 20 mg/kg/day, asymmetric pelvis at fetal incidence of 9.8% exceeded incidence reported for available historical control data; litter incidence was 56% (historical litter incidence not available); neither was statistically significant. This was considered an equivocal finding and a second study in rabbits was conducted and no pelvic abnormalities were noted. There was also an increased fetal and litter incidence of thickened ribs at costal cartilage (13.7% vs. 1.1%, controls) was considered of uncertain toxicological significance at 20 mg/kg/day. No effects on pup weight were observed. The developmental toxicity NOAEL is greater than 20 mg/kg/day for this study because the asymmetric pelvis seen in this study was not seen at all even at higher doses in the 1997 rabbit study. The developmental NOAEL of 20 mg/kg/day and the LOAEL of 40 mg/kg/day will be based on the 1997 rabbit study. The LOAEL is based on supernumerary ribs and decreases in fetal weight.

In light of the equivocal findings in the 1986 study, HED recommended a new study to confirm or otherwise resolve the significance of these finding (refer to memo from R. Gardner to P. Hundemann dated April 15, 1987, HED Document No.: 005840).

The 1997 study is considered to be more appropriate to satisfy the developmental toxicity study in rabbits and the 1986 study is considered to have technical problems such as: 1) current standards as used in the 1997 study require that dosing be continued to day 28 of gestation, in the 1986 study dosing was on days 18-19 only; 2) there was the possibility of poor animal health as indicated by intercurrent infection and some animals were sacrificed *in extremis* (two controls and one in each dose group); 3) in the 1986 study at gestation day 29, Caesarian sectioning revealed animals with intrauterine infection, there were also abortions in the 1986 study since none were seen in the 1997 study; 4) fewer animals (only 9 to 12) were pregnant and had live fetuses available for examination in the 1986 study, the 1997 study had more pregnant does (16 to 19) and live fetuses; and 5) there were data reporting problems in correlating the effects reported to individual fetuses. Because of these reasons, the HIARC determined that this study should be classified as unacceptable/nonguideline.

Second Study (1997).

In the 1997 study, (MRID 45051001) thiophanate-methyl (97.28% purity) was administered to groups of 20 New Zealand White Rabbits by gavage in a 1% aqueous methyl cellulose vehicle (at a rate of 10 mL/kg) at dose levels of 0, 5, 10, 20 or 40 mg/kg/day on gestation days 6 to 28. The rabbits were sacrificed on day 29 and the does were subjected to uterine examination and the pups subjected to external, visceral and skeletal examination.

At 20 mg/kg/day there was **decreased body weight *gain*** (56%, < 0.05) for the interval days 12-15 and body weight gain was decreased 13% for the entire dosing period. At 40 mg/kg/day, body weight gain was decreased and there was actual body weight loss for the interval days 6-9 (.i.e.. the controls gained 80±40 g while the 40 mg/kg/day dose group actually lost 110±100 g). Final (day 29) body weight of the does in the high dose group was 6% less than the control. **Decreased food consumption** accompanied the decease in body weight with there being 13 to 20% decrease in the 20 mg/kg/day dose group and 24 to 70% decreased in the high dose group. The high dose group also had more does with scant or no feces. There were no abortions.

The LOAEL for maternal toxicity is 20 mg/kg/day based on body weight and food consumption decreases. The NOAEL is 10 mg/kg/day.

At 40 mg/kg/day, there were statistically significant ($p < 0.01$) *increases* in the mean number of ossification sites in the thoracic vertebrae (+3.12%) and ribs-pairs (+3.21%) as well as a *decrease* in lumbar vertebrae (-6%) and the differences were in excess of or less than the historical control range respectively. These conditions were collectively referred to as an *increase* in "**supernumerary ribs**" by the study author and were described as a reversible condition. There were also decreases (not statistically significant) in fetal weight (-9.6% for males and -6.6% for females). **The LOAEL is 40 mg/kg/day based on supernumerary ribs and decrease in fetal weight. The NOAEL is 20 mg/kg/day.**

Classification: This study is classified as **Acceptable - Guideline** and satisfies the requirement for a series 83-3 developmental toxicity study in rabbits.

(2) Reproductive Toxicity:

In a **2-generation reproductive toxicity study**, male and female Sprague-Dawley Crl:CD(SD)BR rats (MRIDs 42899101 to 42899105, 43624401) were fed the test material [Topsin-M (95.9% a.i.)] in the diet at concentrations of 0, 200, 630, or 2000 ppm (calculated to be 0, 13.7, 43.3 or 138.9 mg/kg/day for males and 0, 15.5, 54.0 or 172.0 mg/kg/day for females). Twenty-five animals/sex/dose/generation were selected for testing. The P generation animals were given test or control diet for 14 weeks (98 days) then mated to produce the F_1 animals. Approximately 14 weeks after weaning of all F_1 offspring, selected F_1 animals were mated within the same dose group for a maximum of 21 days (sibling matings were avoided) to produce the F_{2a} generation. After weaning of the F_{2a} pups, F_1 animals were maintained for 6 weeks and mated again to the same partner to produce the F_{2b} offspring. The second mating of the F_1 animals was performed due to a high, unexplained death rate in the F_{2a} treated and control pups during lactation. All animals were exposed to test material, either in the diet or during lactation, until sacrifice.

No clinical signs of toxicity or mortalities in the parental animals of either generation were attributable to treatment. There were no significant differences in body weights of the P generation high-dose males and the F_1 mid- and high dose males during the pre-mating periods when compared to controls, however, there was a slight dose-related reduction in body weights throughout the study. The premating period (days 1-43) body weight gains in the 630 and 2000 ppm F_{1b} males were less than controls: 56 and 55% of the control value, respectively. These were considered to be borderline significant because the changes were in the range of 5% of the total bodyweight. In females, although there were some decreases in bodyweight and bodyweight gain during gestation, these were not consistent across generations and/or litters and were thus not biologically significant. High-dose P generation males and females and high-dose F_1 males had significantly ($p \leq 0.05$) increased liver and thyroid weights and high-dose F_1 females had increased thyroid weights when compared to controls. Increased organ weights correlated with statistically significant increases in hepatocellular hypertrophy and thyroid follicular cell hyperplasia/hypertrophy in the high dose group. Generally, minimal to slight hepatocellular

hypertrophy and thyroid follicular cell hypertrophy and hyperplasia were observed in both the low and mid-dose P generation males. These effects were observed in the F_1 generation but appeared in fewer animals and were less severe. In females, these effects were considerably less. **Therefore, the NOAEL for systemic toxicity is <200 ppm (13.7 mg/kg/day) based on hepatocellular hypertrophy and thyroid follicular cell hypertrophy/hyperplasia at all dose levels and decreased body weight gains in males and increased liver and thyroid weights in both sexes at the highest dose level. This LOAEL is considered to be a borderline NOAEL/LOAEL because the effects on the thyroid and liver at 200 ppm were minimal and they were less in the succeeding generation**.

No treatment-related effects were noted on the reproductive performance indices of either generation. Mean litter sizes, survival indices, and sex ratios were not different between treated and control groups for the F_1 and F_{2b} offspring. Due to a high rate of death in both the treated and control F_{2a} pups, a second mating of the F_1 animals was made to produce the F_{2b} offspring. Deaths of the F_{2a} pups did not appear to be treatment-related as controls were equally affected and the result was not repeated after the second mating. No statistically significant differences occurred in mean pup weights from any treated group at any time during lactation of the F_1 or F_{2a} pups. However, F_{2b} pups gained less weight than controls with day 21 body weights of 630 and 2000 ppm group males and females being 88% of the control value. When mean pup weights for the F_{2b} litters were analyzed by covariance analysis (ANCOVA) to account for the number of pups per litter, significantly lower weights as compared to control were seen for the 630 ppm males and females on day 1 ($p \leq 0.01$), 2000 ppm males on day 21 ($p \leq 0.05$), and 630 and 2000 ppm females on day 21 ($p \leq 0.05$). Decreased F_{2b} pup weights were not coincident with reduced dam weights since high-dose dams actually gained slightly more than controls during lactation. **Therefore, the LOAEL for offspring toxicity is 630 ppm (43.3 mg/kg/day) based on reduced body weights of the F_{2b} pups during lactation. The corresponding NOAEL is 200 ppm (13.7 mg/kg/day). This LOAEL is also considered to be borderline because the decrease in pup weights was minimal. The reproductive toxicity LOAEL is >2000 ppm (138.9 mg/kg/day) and the NOAEL is $\geq$2000 ppm.**

This study is classified as Acceptable and satisfies the guideline requirements for a multigeneration reproduction feeding study (83-4).

In a **3-generation reproductive toxicity study** (MRID 00117870), 10 male and 20 female CD rats/dose group were administered thiophanate-methyl (purity not stated) at dietary concentrations of 0, 40, 160 or 640 ppm (equivalent to approximately 0, 2, 8 or 32 mg/kg/day, as estimated using ppm in diet and a standard factor of 0.05 to convert ppm to mg/kg/day in rats). Administration of treated diets to weanling F0, F1b and F2b parental animals was initiated 60 days prior to mating. Each male was cohabited with 2 females. Two matings were conducted for each of the 3 generations. Control and high dose F3b offspring were also examined microscopically for soft tissue and skeletal abnormalities (10/sex each) and weights of many major organs were also measured.

Parental/reproductive toxicity: There were no treatment-related effects on mortality, clinical signs,

body weight, food consumption or gross findings in the parental animals. No effects on reproductive parameters were observed. **The LOAEL for parental systemic/reproductive toxicity is >640 ppm (32 mg/kg/day). The NOAEL is ≥640 ppm.**

Offspring toxicity: At 640 ppm, slightly decreased mean litter weights were observed in both mating of all 3 generations except for the F3a litters (-3.7% to -15.4% less than controls; not statistically significant). This was attributed in part to slightly lower litter sizes as well as lower individual pup weights. These decreases tended to continue throughout lactation in most groups (at lactation day 21, -5.5% to -17% less than controls; F1b and F3a weights not decreased). There were no treatment-related effects on viability indices or gross findings. F3b animals examined microscopically showed no treatment-related abnormalities and no organ weight changes. **The LOAEL for offspring toxicity is 640 ppm (32 mg/kg/day), based on slight but consistently observed decreases in mean litter weights (5 of 6 matings). The NOAEL is 160 ppm (8 mg/kg/day).**

This study is classified **Unacceptable (§83-4)-Upgradable**. Although the study appeared to have been appropriately conducted, purity of the test material was not given in the report. However, an acceptable rat multigeneration reproduction study was previously submitted (MRID 42899101 through 42899105 and 43624401; reviewed in HED Doc. No. 011748) which satisfies the guideline requirement for 83-4. No further information is therefore needed at this time to satisfy this requirement.

D. Determination of Susceptibility

No quantitative or qualitative evidence for increased susceptibility was seen following *in utero* exposure to rats and rabbits or following pre/post natal exposure to rats

No developmental toxicity was seen at the highest dose tested following either gavage (1000 mg/kg/day, Limit-Dose) or dietary (2500 ppm, 163 mg/kg/day) administrations to pregnant rats. In the 1986 study conducted in New Zealand White rabbits, the maternal toxicity NOAEL was 6 mg/kg/day and the LOAEL was 20 mg/kg/day based on decreased body weight/food consumption and abortions. This study indicated an *apparent* developmental toxicity as manifested by increased incidences of asymmetric pelvis and thickened ribs at costal cartilage. However, these apparent and equivocal effects were not seen in s second more definitive rabbit developmental toxicity study (see below).

In the 1997 study also conducted in New Zealand White rabbits at a testing laboratory in the USA, there was no evidence of increased susceptibility. The developmental NOAEL (20 mg/kg/day) and LOAEL (40 mg/kg/day) was higher than the maternal NOAEL (10 mg/kg/day) and LOAEL (20 mg/kg/day). The maternal LOAEL was based on decreases in body weight gain and food consumption. The developmental LOAEL was based on supernumerary ribs.

The HIARC concluded that the pelvic abnormalities seen in the 1986 study are ambiguous and not a concern for increased susceptibility to infants and children based on the following factors:
1) pelvic abnormalities were not seen in the repeat study designed to assess the equivocal finding in the original study and this study employed higher doses (thus a lack of confirmation of the

earlier findings); 2) the incidents (fetal/litter) do not have a dose-response relationship and statistical significance was not attained; 3) ossification of the pelvic bone occurs late in development and therefore the "timing" of sacrifice and examination associated with determining the incidence of "asymmetric pelvis" makes slight differences in this condition of questionable biological significance; and 4) the findings of "asymmetric pelvis" could be an artifact of the staining procedures used at the testing laboratory.

At the April 8, 1999 HIARC meeting, a determination was made to request a developmental neurotoxicity study (DNT) based on the following factors: 1) the concern for increased susceptibility seen in the 1986 rabbit study; 2) the uncertainty regarding the data gap for a developmental toxicity study in rats; 3) data gaps for the acute and subchronic neurotoxicity studies; 4) the concern for thiophanate methyl-induced toxicity in the thyroid glands; and 5) structure activity related toxicity with benomyl and carbendazim.

However, at the current meeting (September 26, 2000), the HIARC decided to place the DNT study in "reserve" status for the reasons listed in Part E below. A final decision on this study will be made after the evaluation of the results of factors listed below.

Two reproductive toxicity studies have been submitted for thiophanate-methyl. In the 2-generation study in rats conducted in 1993, there was no evidence of increased susceptibility of offspring: the parental/systemic LOAEL was 13.7 mg/kg/day, the lowest dose tested, based on slight liver and thyroid effects primarily in P males and to a lesser extent in F1 animals. The offspring LOAEL was 43.3 mg/kg/day, based on decreased F2b pup weights during lactation and the NOAEL was 13.7 mg/kg/day. No reproductive toxicity was observed at any dose. In an earlier 3-generation rat study from 1972, parental or reproductive toxicity was not observed at any dose tested (up to an estimated 32 mg/kg/day). Slight (not statistically significant) decreases in litter weights were observed in most litter groups at 32 mg/kg/day. This decrease was marginal and due, in part, to reduced litter size. The difference in parental NOAELs/LOAELs may be due to the apparent lack of microscopic examination of parental animals in the earlier study.

E. Recommendation for a Developmental Neurotoxicity Study

The HIARC concluded to place the developmental neurotoxicity study (DNT) in rats in **"reserve"** status based on the following factors:

1. *Review/evaluation of the acute and subchronic neurotoxicity studies in rats.* Evidence of potential neurotoxicity of thiophanate-methyl in the toxicology database were limited to the chronic oral toxicity study in the dog, in which tremors were observed at the highest dose tested (200 mg/kg/day) in a few dogs between days 1 and 17. No tremors were seen after day 17 through study termination. Tremors were not seen in any rat studies or in the rabbit developmental toxicity studies.

2. *Development of a policy on the need for a DNT for pesticides that cause toxicity in the thyroid glands*. Thiophanate-methyl appears to disrupt thyroid-pituitary homeostasis as demonstrated in the subchronic and chronic dietary studies in rats and dogs. The extent to which these effects occur *in utero* or during lactation and

might cause subtle alterations in the development or function of the nervous system are unclear and should be evaluated. However, the HIARC was not certain that the current DNT protocol would provide an adequate evaluation to address this concern.

3. *Receipt/evaluation of the results of the DNT study requested for benomyl and the common metabolite, MBC.* Although both thiophanate-methyl and benomyl share a common metabolite, they appear to cause different developmental effects. Whereas prenatal exposure to benomyl is reported to cause ocular and cerebral malformations in rats at doses lower than those causing maternal toxicity, these effects were not observed with thiophanate-methyl. No developmental toxicity was observed in the rabbit following prenatal exposure to benomyl or thiophanate methyl.

4. *There was no evidence increased susceptibility following in utero exposures to rats and rabbits and pre/post natal exposures to rats.*

VI. HAZARD CHARACTERIZATION

Thiophanate-methyl is a systemic protectant fungicide of the benzimidazole class, and is a carbamate. The toxicology database for thiophanate-methyl is not complete at this time. In addition the HIARC determined that rat acute and subchronic neurotoxicity screening studies and a rat developmental neurotoxicity study are required. Confidence in the available studies is high.

The toxicology profile indicates that the liver and thyroid are the target organs of thiophanate-methyl-induced toxicity. In the subchronic and chronic toxicity studies on rats and dogs, liver and thyroid enlargement were observed, along with hepatocellular hypertrophy and follicular cell hypertrophy/hyperplasia. Alterations in the levels of thyroid hormones (increased TSH, decreased T3/T4) were also reported. The findings of the dietary studies, together with the results of a mechanistic study evaluating short-term liver and thyroid effects in treated rats and mice, provide evidence that thiophanate-methyl alters thyroid-pituitary homeostasis, probably primarily by increasing activities of liver microsomal enzymes, particularly UDP-glucuronosyltransferase, and possibly to a lesser extent by inhibition of thyroid peroxidase activity.

Following long-term dietary administration, thiophanate-methyl caused an increased incidence in thyroid follicular cell adenoma and carcinoma in male F344 rats and an increase in adenoma in female rats. The incidence was only statistically significant in males at the HDT, which exceeded the maximum tolerated dose based on excessive mortality. An increasing trend was observed in both sexes, although in the next-to-highest dose in males and at the highest two dose levels in females, only a few tumors were observed. In male and female CD-1 mice, the incidence of hepatocellular adenoma was significantly increased at the highest two dose levels and also showed an increasing trend.

Thiophanate-methyl showed evidence of genotoxicity in *in vivo* mouse micronucleus assay and *in vitro* BALB/c3T3 cell transformation assays (published studies). The available data indicate that thiophanate-methyl is aneugenic. MBC, the common metabolite with benomyl, also has been shown to be aneugenic.

There was no quantitative or qualitative evidence of increased susceptibility for *in utero* exposure to thiophanate-methyl in the rat or rabbit developmental toxicity studies. There was no evidence of reproductive toxicity or increased susceptibility to offspring following pre/post natal exposures in the 2 or 3- generation rat reproductive toxicity studies.

At this time no neurotoxicity screening studies in the rat have been submitted. The only study in which potential indications of neurotoxicity were reported was the chronic feeding study in the dog, in which tremors were observed post-dosing in almost all animals at the highest dose tested (200 mg/kg/day) on day 1 and sporadically in a few animals through day 27.

VII. DATA GAPS

The HIARC recommended that the following additional studies be conducted: (1) 870.6100, acute neurotoxicity screening study in the rat; (2) 870.6100, subchronic neurotoxicity screening study in the rat; and (3) 870.3465, 90-day rat inhalation study. A 90-day rat inhalation study was requested because the unacceptable 14-day inhalation study showed possible respiratory effects from thiophanate methyl exposure at lower concentrations than those associated with developmental effects, and because there are inhalation exposures associated with the residential (i.e., turf) and agricultural uses that need to be evaluated in the risk assessment. The developmental neurotoxicity study in rats is placed in reserve status pending review of the acute and subchronic neurotoxicity studies.

VIII. ACUTE TOXICITY ENDPOINTS:

Acute Toxicity of Thiophanate-Methyl (tech. a.i.)				
Guideline No.	**Study Type**	**MRID #**	**Results**	**Toxicity Category**
81-1	Acute Oral, Rat	41644301	LD_{50} = >5000 mg/kg, both sexes	IV
81-2	Acute Dermal, Rabbit	41644302	LD_{50} = >2000 mg/kg, both sexes	III
81-3	Acute Inhalation, Rat	41482804	LC_{50} = 1.7 mg/L, males 1.9 mg/L, females	III
81-4	Primary Eye Irritation, Rabbit	40095501	Slight ocular irritant	IV
81-5	Primary Skin Irritation, Rabbit	40095502	Not a dermal irritant	
81-6	Dermal Sensitization, Guinea Pig	41482805	Is a dermal sensitizer	

N/A No data available

IX. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

Thiophanate-Methyl: Revisions to the Toxicology Endpoint Selection Made at the **September 26, 2000** HIARC revisit.

Scenario	Dose	Endpoint	Study
Acute Dietary (Females 13 -50)	Developmental NOAEL = 20 mg/kg/day UF = 100	Supernumerary ribs at 40 mg/kg/day.	1997 Developmental Toxicity - Rabbit
	Acute RfD (females 13+) = 0.20 mg/kg/day		
Acute Dietary (General Population including infants and children)	NOAEL = 40 mg/lg/day UF = 100	Tremors in 7 of 8 dogs 2-4 hours postdosing at 100 mg/kg/day.	Chronic Toxicity-Dog
	Acute RfD (general Population) = 0.40 mg/kg/day		
Chronic Dietary	NOAEL = 8 mg/kg/day UF = 100	Decreased body weight and thyroid effects at 40 mg/kg/day.	Chronic Toxicity-Dog
	Chronic RfD (all populations) = 0.08 mg/kg/day		
Incidental Oral Ingestion Short & Intermediate Term	Maternal NOAEL = 10 mg/kg/day UF = 100	Decreased maternal body weight and food consumption at 20 mg/kg/day.	Developmental Toxicity - Rabbit
Dermal Absorption	7%	Based on comparison of the LOAEL for the 1997 rabbit developmental toxicity of 20 mg/kg/day and the LOAEL foe the rabbit 21-day dermal study of 300 mg/kg/day.	
Dermal, Short and Intermediate -Term**	NOAEL = 100 mg/kg/day	Decreased body weight (28%) and consumption (15%) at 300 mg/kg/day.	21-day Dermal Toxicity - Rabbits
Dermal, Long Term**	Oral NOAEL* = 8 mg/kg/day	Decreased body weight and thyroid effects at 40 mg/kg/day.	Chronic Toxicity-Dog
Inhalation, Short and Intermediate-Term**	Oral NOAEL* = 10 mg/kg/day	Decreased maternal body weight and food consumption at 20 mg/kg/day.	Developmental Toxicity - Rabbit
Inhalation, Long Term**	Oral NOAEL* = 8 mg/kg/day	Decreased body weight and thyroid effects at 40 mg/kg/day.	Chronic Toxicity-Dog

*Since oral values were selected dermal (7%) and inhalation (100%) absorption factors must be used for route to route extrapolation. ** MOE for occupational dermal and inhalation risk assessment is 100. The MOEs for residential exposure will be determined by FQPA Safety Factor Committee.